

Claims:

1. A kit for detecting a target nucleotide sequence in a sample, the kit comprising:
- (a) a support surface comprising one or more immobilized capture oligonucleotides;
 - (b) a detection probe comprising an oligonucleotide tag, a target complement and a
5 detection oligonucleotide;
 - (c) an amplification template;
 - (d) a nucleic acid ligase;
 - (e) a nucleic acid polymerase; and
 - (f) a detection reagent comprising a label and a nucleic acid sequence.

10 2. The kit according to claim 1, further comprising an anchoring reagent comprising an oligonucleotide tag and an anchoring oligonucleotide.

15 3. The kit according to claim 2, wherein the anchoring reagent is immobilized on the support surface.

4. The kit of according to claim 2 or 3, wherein the anchoring oligonucleotide is about 10 to about 30 nucleic acids in length.

20 5. The kit of according to any of claims 2 to 4, wherein the anchoring oligonucleotide comprises 5'-AAGAGAGTAGTACAGCA-3' (SEQ ID NO:1669).

25 6. The kit according to any of claims 2 to 5, wherein the amplification template comprises a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, and an internal nucleotide sequence capable of hybridizing to a complement of the anchoring oligonucleotide of the anchoring reagent, wherein the 5' and 3' terminal nucleotide sequences of the amplification template do not overlap with the internal sequence.

7. The kit according to claim 6, wherein the amplification template comprises a 5' terminal phosphate group.
8. The kit according to any of claims 1 to 7, wherein the amplification template is about 53 to about 61 nucleotides in length.
9. The kit according to any of claims 6 to 8, wherein the sum of the length of the 5' and 3' terminal sequences is about 14 to about 24 nucleotides in length.
10. The kit according to claim 9, wherein the sum of the length of the 3' and 5' terminal sequences is about 14 to about 19 nucleotides in length.
11. The kit according to any of claims 1 to 10, wherein the amplification template comprises a 5' terminal sequence of 5'-GTTCTGTC-3' (SEQ ID NO: 1666) and 3' terminal sequence of 5'-GTGTCTA-3' (SEQ ID NO: 1667).
12. The kit according to any of claims 1 to 10, wherein the amplification template comprises a nucleotide sequence of:
- a) 5'-CAGTGAATGCGAGTCCGTCTAAG-3' (SEQ ID NO:1668);
- b) 5'-AAGAGAGTAGTACAGCA-3' (SEQ ID NO:1669);
- c) 5' -
GTTCTGTCATATTTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAGTACAGCAA
GAGTGTCTA-3' (SEQ ID NO:1670); or
- d) 5'-
GCTGTGCAATATTTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAGTACAGCAA
GAGCGTCGA-3' (SEQ ID NO:1671).
13. The kit according to claim 1, wherein the amplification template comprises a circular amplification template.

14. The kit according to any of claims 1 to 13, wherein the detection probe comprises a single stranded DNA oligonucleotide tag, a single stranded RNA target complement and a single stranded DNA detection oligonucleotide.
- 5 15. The kit according to claim 2, wherein the anchoring reagent comprises a single stranded DNA oligonucleotide tag and a single stranded DNA anchoring sequence.
16. The kit according to claim 14 or 15, further comprising an RNase.
- 10 17. The kit according to claim 6, wherein the detection oligonucleotide of the detection probe comprises a first sequence complementary to the 5' terminal sequence of the amplification template and an adjacent second sequence complementary to the 3' terminal sequence of the amplification template.
- 15 18. The kit according to any of claims 1 to 17, wherein the nucleic acid sequence of the detection reagent comprises
- a) a sequence with at least 90% sequence identity to 14 or 15 contiguous nucleotides of: 5'-CAGTGAATGCGAGTCCGTCT-3' (SEQ ID NO:1672);
 - b) the sequence 5'-CAGTGAATGCGAGTCCGTCT-3' (SEQ ID NO:1672); or
 - 20 c) the sequence 5'-CAGTGAATGCGAGTCCGTCTAAG-3' (SEQ ID NO:1668).
19. The kit according to any of claims 1 to 18, wherein the label of the detection reagent comprises an electrochemiluminescent (ECL) label.
- 25 20. The kit according to any of claims 1 to 19, wherein the support surface comprises a carbon-based electrode.
21. The kit according to any of claims 1 to 20, wherein the support surface comprises a carbon ink electrode.

22. The kit according to any of claims 1 to 21, wherein the support surface comprises a multi-well plate assay consumable, and wherein each well of the plate comprises a carbon ink electrode.
- 5 23. The kit according to any of claims 1 to 22, wherein a plurality of capture oligonucleotides are immobilized on a solid phase support in discrete binding domains to form an array.
24. The kit according to any of claims 1 to 23, wherein the capture oligonucleotides immobilized on the support surface are selected from:
- 10 1. Capture set 1 comprising:
- a. capture oligonucleotides having at least 20 consecutive nucleotides of a sequence selected from SEQ ID Nos: 1-64;
 - b. capture oligonucleotides comprising a sequence having at least 95% identity to a sequence selected from SEQ ID Nos: 1-64;
 - 15 c. capture oligonucleotides having at least 20 consecutive nucleotides of a sequence having at least 95% identity to as sequence selected from SEQ ID Nos: 1-64;
 - d. capture oligonucleotides comprising a sequence selected from SEQ ID Nos: 1-64; and
 - 20 e. capture oligonucleotides selected from any of (a)-(d); or
2. Capture set 2 comprising:
- f. capture oligonucleotides comprising a sequence having at least 20 consecutive nucleotides of a sequence selected from SEQ ID Nos: 1-10;
 - g. capture oligonucleotides comprising a sequence having at least 95% identity to a sequence selected from SEQ ID Nos: 1-10;
 - 25 h. capture oligonucleotides having at least 20 consecutive nucleotides of a sequence having at least 95% identity to as sequence selected from SEQ ID Nos: 1-10;
 - i. capture oligonucleotides comprising a sequence selected from SEQ ID Nos: 1-10; and
 - 30 j. capture oligonucleotides selected from any of (f)-(i).

25. A kit for detecting a target nucleotide sequence in a sample, the kit comprising:
- (a) a support surface comprising immobilized capture oligonucleotide;
 - (b) an anchoring reagent comprising an oligonucleotide tag and an anchoring oligonucleotide;
 - 5 (c) a targeting probe comprising a single stranded oligonucleotide tag and a first nucleic acid sequence that is complementary to a first region of the target nucleotide sequence in the sample;
 - (d) a detection probe comprising a detection oligonucleotide and a second nucleic acid sequence that is complementary to a second region of the target nucleotide sequence, wherein the first nucleic acid sequence of the targeting probe and
10 second nucleic acid sequence of the detection probe are complementary to adjacent sequences of the target nucleotide;
 - (e) a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, a first internal nucleotide sequence capable of hybridizing to a complement of the anchoring sequence of the anchoring reagent and a second internal nucleotide sequence capable of hybridizing to a complement of the nucleic acid sequence of the detection reagent, wherein the 5' and 3' terminal nucleotide sequences of the
15 amplification template do not overlap with the first and second internal sequences;
 - (f) a nucleic acid ligase;
 - (g) a nucleic acid polymerase; and
 - (h) a detection reagent comprising an electrochemiluminescent (ECL) label and a
20 nucleic acid sequence.
- 25
26. A kit according to any of claims 1 to 25, further comprising a detection mixture comprising a linear amplification template and one or more additional components, selected from: ligation buffer, adenosine triphosphate (ATP), bovine serum albumin (BSA), Tween 20,
30 T4 DNA ligase, and combinations thereof.

27. A kit according to claim 26, wherein the detection mixture comprises one or more components for rolling circle amplification selected from BSA, buffer, deoxynucleoside triphosphates (dNTP), Tween 20, Phi29 DNA polymerase, or a combination thereof.

28. A method of detecting a target oligonucleotide comprising a target nucleic acid sequence in a sample, the method comprising:

(a) contacting the sample with a detection probe comprising an oligonucleotide tag, a target complement and a detection oligonucleotide under conditions in which the target complement hybridizes to the target nucleic acid sequence of the target oligonucleotide to form a reaction product;

(b) contacting a support surface on which a capture oligonucleotide is immobilized with a mixture containing the reaction product under conditions in which the oligonucleotide tag of the reaction product hybridizes to the capture oligonucleotide to form an immobilized detection complex;

(c) contacting the immobilized detection complex with a detection mixture comprising an amplification template;

(d) amplifying the amplification template to form an amplicon comprising one or more nucleic acid sequences comprising detection labeling sites;

(e) contacting the amplicon with a detection reagent comprising a label and a nucleic acid sequence that is complementary to the detection labeling sites under conditions in which the nucleic acid sequence of the detection reagent hybridizes to the detection labeling sites of the amplicon; and

(f) detecting the label bound to the detection labeling sites.

29. A method of detecting a target oligonucleotide comprising a target nucleic acid sequence in a sample, the method comprising:

(a) contacting the sample with:

(i) a detection probe comprising an oligonucleotide tag comprising a single stranded DNA sequence, a target complement comprising a single stranded RNA sequence and a detection oligonucleotide comprising a single stranded DNA sequence; and

(ii) an anchoring reagent comprising an oligonucleotide tag comprising a single stranded DNA sequence and an anchoring sequence comprising a single stranded DNA sequence,

wherein the target complement of the detection probe hybridizes to the target nucleic acid sequence of the target oligonucleotide to form a reaction product comprising the oligonucleotide tag, a double stranded RNA duplex comprising the target nucleic acid sequence of the target oligonucleotide and the target complement;

- (b) contacting a support surface comprising one or more electrodes on which a plurality of capture oligonucleotides are immobilized in discrete binding domains with a mixture comprising the reaction product under conditions in which the oligonucleotide tag of the reaction product hybridizes to the capture oligonucleotides to form a detection complex on the support surface;
- (c) contacting the support surface with a RNase to digest single stranded RNA of unbound detection probe;
- (d) contacting the immobilized detection complex with a detection mixture comprising a rolling circle amplification (RCA) template and a polymerase;
- (e) amplifying the template by RCA to form an extended sequence attached to the detection complex, wherein the extended sequence comprises multiple nucleic acid sequences comprising detection labeling sites;
- (f) contacting the extended sequence with a detection reagent comprising an electrochemiluminescent (ECL) label and a nucleic acid sequence that is complementary to the detection labeling sites of the extended sequence under conditions in which the nucleic acid sequence of the detection reagent hybridizes to the detection labeling sites; and
- (g) detecting the ECL label bound to the extended sequence by contacting the ECL label with an ECL read buffer comprising an ECL co-reactant, and applying an electrical potential to the electrodes.

30. A method of detecting a target nucleotide sequence in a sample, the method comprising:
- (a) contacting the sample with a mixture comprising:

- (i) a targeting probe comprising a single stranded oligonucleotide tag and a first nucleic acid sequence that is complementary to a first region of the target nucleotide sequence in the sample; and
- (ii) a detecting probe comprising a detection oligonucleotide and a second nucleic acid sequence that is complementary to a second region of the target nucleotide sequence,
- wherein the first nucleic acid sequence of the targeting probe and second nucleic acid sequence of the detecting probe are complementary to adjacent nucleic acid sequences of the target oligonucleotide;
- (b) incubating the mixture comprising the target oligonucleotide, targeting probe and detecting probe in the presence of a nucleic acid ligase under conditions in which the targeting probe and the detecting probe bind to their corresponding nucleotide sequences of the target oligonucleotide and the nucleic acid ligase ligates the targeting and detecting probes to form a reaction product comprising the oligonucleotide tag and detection oligonucleotide;
- (c) contacting a support surface on which a capture oligonucleotide is immobilized with the mixture comprising the reaction product under conditions in which the oligonucleotide tag of the reaction product hybridizes to the capture oligonucleotide to form an immobilized detection complex;
- (d) contacting the immobilized detection complex with a detection mixture comprising an amplification template;
- (e) amplifying the amplification template to form an amplicon comprising one or more nucleic acid sequences comprising detection labeling sites;
- (f) contacting the amplicon with a detection reagent comprising a label and a nucleic acid sequence that is complementary to the detection labeling sites under conditions in which the nucleic acid sequence of the detection reagent hybridizes to the detection labeling sites; and
- (g) detecting the label bound to the support surface.